

What is claimed:

1. A method for determining hemoglobin oxygen saturation in a tissue *in vivo*; said method comprising the steps of:

(a) measuring the visible reflectance spectrum or the visible absorbance spectrum of the tissue *in vivo* over at least a portion of the range of wavelengths between 530 nm and 584 nm;

(b) determining one or more of the values x, y, and z, wherein:

(i) x is proportional to the integral of { the reference stratum minus the measured visible spectrum } over at least a portion of region I;

(ii) y is proportional to the integral of { the measured visible spectrum minus the reference stratum } over at least a portion of region II;

(iii) z is proportional to the integral of { the reference stratum minus the measured visible spectrum } over at least a portion of region III;

(iv) region I is the region of the visible spectrum between the hemoglobin isosbestic point at about 530 nm and the hemoglobin isosbestic point at about 545 nm;

(v) region II is the region of the visible spectrum between the hemoglobin isosbestic point at about 545 nm and the hemoglobin isosbestic point at about 570 nm;

(vi) region III is the region of the visible spectrum between the hemoglobin isosbestic point at about 570 nm and the hemoglobin isosbestic point at about 584 nm;

- (vii) the reference stratum comprises a sequence of three linear segments: a first linear segment connecting the visible reflectance or absorbance of hemoglobin at the isosbestic point at about 530 nm to the visible reflectance or absorbance of hemoglobin at the isosbestic point at about 545 nm; a second linear segment connecting the visible reflectance or absorbance of hemoglobin at the isosbestic point at about 545 nm to the visible reflectance or absorbance of hemoglobin at the isosbestic point at about 570 nm; and a third linear segment connecting the visible reflectance or absorbance of hemoglobin at the isosbestic point at about 570 nm to the visible reflectance or absorbance of hemoglobin at the isosbestic point at about 584 nm;

whereby:

- (viii) larger values of  $x$ ,  $y$ , or  $z$  correspond to higher hemoglobin oxygen saturation in the tissue *in vivo*, and lower values of  $x$ ,  $y$ , or  $z$  correspond to lower hemoglobin oxygen saturation in the tissue *in vivo*.

2. The method of Claim 1, wherein:

- (i)  $x$  is proportional to the integral of { the reference stratum minus the measured visible spectrum } over region I;
- (ii)  $y$  is proportional to the integral of { the measured visible spectrum minus the reference stratum } over region II; and
- (iii)  $z$  is proportional to the integral of { the reference stratum minus the measured visible spectrum } over region III.

3. The method of Claim 2, additionally comprising the step of determining the sum  $S = x + y + z$ ; whereby larger values of  $S$  correspond to higher hemoglobin oxygen saturation in the tissue *in vivo*, and lower values of  $S$  correspond to lower hemoglobin oxygen saturation in the tissue *in vivo*.
4. The method of Claim 3, wherein:
- (i) the value of  $x$  is corrected by a factor proportional to the integral of { the reference stratum minus the baseline } over region I; wherein the baseline corresponds to zero reflectance or zero absorbance;
  - (ii) the value of  $y$  is corrected by a factor proportional to the integral of { the reference stratum minus the baseline } over region II; and
  - (i) the value of  $z$  is corrected by a factor proportional to the integral of { the reference stratum minus the baseline } over region III.
5. The method of Claim 1, wherein:
- (i) the value of  $x$  is corrected by a factor proportional to the integral of { the reference stratum minus the baseline } over the same portion of region I that is used to determine  $x$ ; wherein the baseline corresponds to zero reflectance or zero absorbance;
  - (ii) the value of  $y$  is corrected by a factor proportional to the integral of { the reference stratum minus the baseline } over the same portion of region II that is used to determine  $y$ ; and
  - (i) the value of  $z$  is corrected by a factor proportional to the integral of { the reference stratum minus the baseline } over the same portion of region III that is used to determine  $z$ .

6. The method of Claim 1, wherein the tissue is a blood vessel.
7. The method of Claim 1, wherein the tissue is skin.
8. The method of Claim 1, wherein the tissue is a retina or a blood vessel in a retina.
9. A method comprising repeating the method of Claim 1 at a plurality of locations in the tissue, and plotting the inferred hemoglobin oxygen saturation levels in the tissue as a function of location.
10. A plot of inferred hemoglobin oxygen saturation levels in a tissue, wherein said plot is produced by the method of Claim 9.
11. A method for diagnosing abnormal hemoglobin oxygen saturation in a tissue, said method comprising conducting the method of Claim 1 on the tissue, and identifying any abnormal values of x, y, or z as indicating abnormal hemoglobin oxygen saturation in the tissue.
12. The method of Claim 11, wherein the diagnosis is conducted in a patient or in a tissue having a condition selected from the group consisting of glaucoma, diabetic retinopathy, hypertension, sickle cell disease, vascular disease, skin disorder, a wound, and a burn.
13. A method to enhance the visual delineation of blood vessels in a tissue, said method comprising conducting the method Claim 9 on the tissue, wherein the plot of hemoglobin oxygen saturation levels as a function of location delineates the location of the blood vessels.